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**WO 03/104218 A1**

**(54) Title:** NF-:B INHIBITORS

**(57) Abstract:** The present invention provides novel compounds and methods for using them to treat diseases with aminothiophene inhibitors of IKK-β phosphorylation of I:B. In so doing these aminothiophene inhibitors block pathological activation of transcription factor NF-:B in which diseases excessive activation of NF-:B is implicated.

**NF-κB Inhibitors****FIELD OF THE INVENTION**

This invention relates in general to a method of inhibiting pathological activation of the transcription factor NF-κB (nuclear factor-κB) using aminothiophene compounds. Such methods are particularly useful for treating diseases in which activation of NF-κB is implicated. More specifically, these methods may be used for inhibiting IKK-β (IκB kinase-β, also known as IKK-2) phosphorylation of IκB (inhibitory protein κB)-which prevents subsequent degradation and activation of NF-κB dimers. Such methods are useful in the treatment of a variety of diseases associated with NF-κB activation including inflammatory and tissue repair disorders, particularly rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease); osteoarthritis, osteoporosis and fibrotic diseases; dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage; autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkin's disease, cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome, and Ataxia Telangiectasia.

**BACKGROUND OF THE INVENTION**

Recent advances in scientific understanding of the mediators involved in acute and chronic inflammatory diseases and cancer have led to new strategies in the search for effective therapeutics. Traditional approaches include direct target intervention such as the use of specific antibodies, receptor antagonists, or enzyme inhibitors. Recent breakthroughs in the elucidation of regulatory mechanisms involved in the transcription and translation of a variety of mediators have led to increased interest in therapeutic approaches directed at the level of gene transcription.

Nuclear factor κB (NF-κB) belongs to a family of closely related dimeric transcription factor complexes composed of various combinations of the Rel/NF-κB family of polypeptides. The family consists of five individual gene products in mammals, RelA (p65), NF-κB1 (p50/ p105), NF-κB2 (p49/ p100), c-Rel, and RelB, all of which can form hetero- or homodimers. These proteins share a highly homologous 300 amino acid “Rel homology domain” which contains the DNA binding and dimerization domains. At the extreme C-terminus of the Rel homology domain is a nuclear translocation sequence important in the transport of NF-κB from the cytoplasm to the nucleus. In addition, p65 and cRel possess potent transactivation domains at their C-terminal ends.

The activity of NF-κB is regulated by its interaction with a member of the inhibitor IκB family of proteins. This interaction effectively blocks the nuclear localization sequence on the NF-κB proteins, thus preventing migration of the dimer to the nucleus. A wide variety of stimuli activate NF-κB through what are likely to be multiple signal transduction pathways. Included are bacterial products (LPS), some viruses (HIV-1, HTLV-1), inflammatory cytokines (TNF $\alpha$ , IL-1), environmental and oxidative stress and DNA damaging agents. Apparently common to all stimuli however, is the phosphorylation and subsequent degradation of IκB. IκB is phosphorylated on two N-terminal serines by the recently identified IκB kinases (IKK- $\alpha$  and IKK- $\beta$ ). Site-directed mutagenesis studies indicate that these phosphorylations are critical for the subsequent activation of NF-κB in that once phosphorylated the protein is flagged for degradation via the ubiquitin-proteasome pathway. Free from IκB, the active NF-κB complexes are able to translocate to the nucleus where they bind in a selective manner to preferred gene-specific enhancer sequences. Included in the genes regulated by NF-κB are a number of cytokines and chemokines, cell adhesion molecules, acute phase proteins, immunoregulatory proteins, eicosanoid metabolizing enzymes and anti-apoptotic genes.

It is well-known that NF-κB plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as TNF, IL-1 $\beta$ , IL-6 and IL-8, cell adhesion molecules, such as ICAM and VCAM, and

inducible nitric oxide synthase (iNOS). Such mediators are known to play a role in the recruitment of leukocytes at sites of inflammation and in the case of iNOS, may lead to organ destruction in some inflammatory and autoimmune diseases.

The importance of NF- $\kappa$ B in inflammatory disorders is further strengthened  
5 by studies of airway inflammation including asthma, in which NF- $\kappa$ B has been shown to be activated. This activation may underlie the increased cytokine production and leukocyte infiltration characteristic of these disorders. In addition, inhaled steroids are known to reduce airway hyperresponsiveness and suppress the inflammatory response in asthmatic airways. In light of the recent findings with  
10 regard to glucocorticoid inhibition of NF- $\kappa$ B, one may speculate that these effects are mediated through an inhibition of NF- $\kappa$ B.

Further evidence for a role of NF- $\kappa$ B in inflammatory disorders comes from studies of rheumatoid synovium. Although NF- $\kappa$ B is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF- $\kappa$ B is present in the nuclei, and hence active, in the cells comprising rheumatoid synovium. Furthermore, NF- $\kappa$ B has been shown to be activated in human synovial cells in response to stimulation with TNF- $\alpha$  or IL-1 $\beta$ . Such a distribution may be the underlying mechanism for the increased cytokine and eicosanoid production characteristic of this tissue. See Roshak, A. K., et al., *J. Biol. Chem.*, 271, 31496-31501 (1996). Expression of IKK- $\beta$  has been shown in synoviocytes of rheumatoid arthritis patients and gene transfer studies have demonstrated the central role of IKK- $\beta$  in stimulated inflammatory mediator production in these cells . See  
20 Aupperle et al. *J. Immunology* 1999; 163:427-433 and Aupperle et al. *J. Immunology* 2001;166:2705-11. More recently, the intra-articular administration of a wild type IKK- $\beta$  adenoviral construct was shown to cause paw swelling while intra-articular administration of dominant-negative IKK- $\beta$  inhibited adjuvant-induced  
25 arthritis in rat. See Tak et al. *Arthritis and Rheumatism* 2001; 44:1897-1907.

The NF- $\kappa$ B/Rel and I $\kappa$ B proteins are also likely to play a key role in neoplastic transformation and metastasis. Family members are associated with cell  
30 transformation *in vitro* and *in vivo* as a result of overexpression, gene amplification ,

gene rearrangements or translocations. In addition, rearrangement and/or amplification of the genes encoding these proteins are seen in 20-25% of certain human lymphoid tumors. Further, NF- $\kappa$ B is activated by oncogenic ras, the most common defect in human tumors and blockade of NF- $\kappa$ B activation inhibits ras mediated cell transformation. In addition, a role for NF- $\kappa$ B in the regulation of apoptosis has been reported, strengthening the role of this transcription factor in the regulation of tumor cell proliferation. TNF, ionizing radiation and DNA damaging agents have all been shown to activate NF- $\kappa$ B which in turn leads to the upregulated expression of several anti-apoptotic proteins. Conversely, inhibition of NF- $\kappa$ B has 5 been shown to enhance apoptotic-killing by these agents in several tumor cell types. As this likely represents a major mechanism of tumor cell resistance to chemotherapy, inhibitors of NF- $\kappa$ B activation may be useful chemotherapeutic agents as either single agents or adjunct therapy. Recent reports have implicated 10 NF- $\kappa$ B as an inhibitor of skeletal cell differentiation as well as a regulator of cytokine-induced muscle wasting (Guttridge et al. *Science*; 2000; 289: 2363-2365.) 15 further supporting the potential of NF- $\kappa$ B inhibitors as novel cancer therapies.

Several NF- $\kappa$ B inhibitors are described in C. Wahl, et al. *J. Clin. Invest.* 101(5), 1163-1174 (1998), R. W. Sullivan, et al. *J. Med. Chem.* 41, 413-419 (1998), J. W. Pierce, et al. *J. Biol. Chem.* 272, 21096-21103 (1997).

20 The marine natural product hymenialdisine is known to inhibit NF- $\kappa$ B. Roshak, A., et al., *JPET*, 283, 955-961 (1997). Breton, J. J and Chabot-Fletcher, M. C., *JPET*, 282, 459-466 (1997).

25 Additionally, patent applications have been filed on aminothiophene inhibitors of the IKK-2, see Callahan, et al., WO 2002030353; Baxter, et al., WO 2001058890, Faull, et al., WO 2003010158; Griffiths, et al., WO2003010163; Fancelli, et al., WO 200198290; imidazole inhibitors of IKK-2, see Callahan, et al., WO 200230423; anilinophenylpyrimidine inhibitors of IKK-2, see Kois, et al., WO 2002046171;  $\beta$ -carboline inhibitors of IKK-2 , see Ritzeler, et al., WO 2001068648, Ritzeler, et al., EP 1134221; Nielsch, et al. DE 19807993; Ritzeler, et al., EP 30 1209158; indole inhbitors of IKK-2, see Ritzeler, et al., WO 2001030774;

benzimidazole inhibitors of the IKK-2, see Ritzeler, et al., DE 19928424; Ritzeler et al, WO 2001000610; aminopyridine inhibitors of IKK-2, see Lowinger, et al, WO2002024679; Murata, et al, WO 2002024693; Murata, et al., WO2002044153;pyrazolaquinazoline inhibitors of IKK-2, see Beaulieu, et al., 5 WO2002028860; Burke et al, WO2002060386, Burke, et al. US 20030022898; quinoline inhibitors of IKK-2, Browner, et al., WO2002041843, Browner, et al., US 20020161004 and pyridylcyanoguanidine inhibitors of IKK-2, see Bjorkling, et al., WO 2002094813, Binderup et al, WO 2002094322 and Madsen, et al., WO 10 200294265.The natural products staurosporine, quercetin, K252a and K252b have been shown to be IKK-2 inhibitors, see Peet, G. W. and Li, J. J. *Biol. Chem.*, 274, 32655-32661 (1999) and Wisniewski, D., et al., *Analytical Biochem.* 274, 220-228 (1999). Synthetic inhibitors of IKK-2 have also been described, see Burke, et al. *J. Biol. Chem.*, 278, 1450-1456 (2003) and Murata, et al., *Bioorg. Med. Chem. Lett.*, 13, 913-198 (2003) have described IKK-2 inhibitors.

15 U.S. Patent No. 3,963,750 describes the preparation of certain aminothiophenes.

### SUMMARY OF THE INVENTION

The present invention involves novel compounds and novel methods of 20 inhibiting the activation transcription factor NF-κB using the present compounds.

An object of the present invention is to provide a method for treating diseases which may be therapeutically modified by altering the activity of transcription factor NF-κB.

Accordingly, in the first aspect, this invention provides a pharmaceutical 25 composition comprising a compound according to Formula I.

In another aspect, this invention provides a method of treating diseases in which the disease pathology may be therapeutically modified by inhibiting phosphorylation and subsequent degradation of IκB by IKK-β.

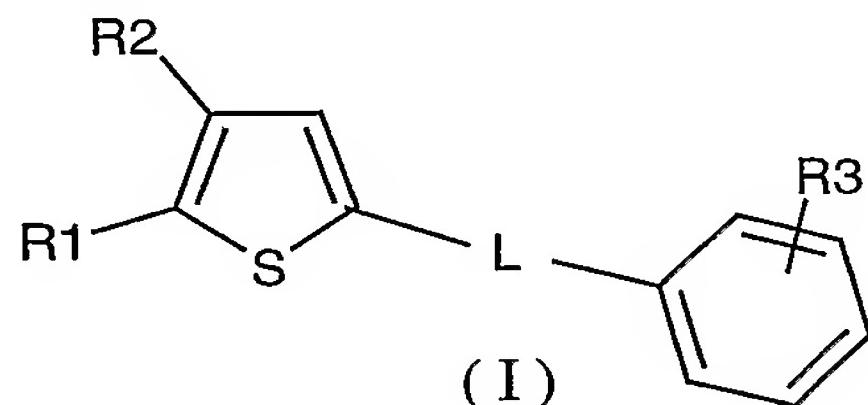
In still another aspect, this invention provides a method of treating diseases 30 in which the disease pathology may be therapeutically modified by inhibiting pathological activation of NF-κB.

In a particular aspect, this invention provides methods for treating a variety of diseases associated with NF- $\kappa$ B activation including inflammatory and tissue repair disorders, particularly rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease) osteoarthritis, 5 osteoporosis and fibrotic diseases, dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage; autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, alkylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkins disease, 10 cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome and Ataxia Telangiectasia.

#### DETAILED DESCRIPTION OF THE INVENTION

15

The compounds of the present invention are selected from Formula (I) herein below:



20 wherein:

R<sub>1</sub> represents NR<sub>4</sub>R<sub>5</sub>;

R<sub>2</sub> represents CONH<sub>2</sub> or SO<sub>2</sub>NH<sub>2</sub>;

R<sub>3</sub> represents up to three substituents selected from the group consisting of halogen, C<sub>1-4</sub>alkyl, NH<sub>2</sub>, CF<sub>3</sub>, OCF<sub>3</sub>, O-alkyl, S-alkyl, CN, CHO, SO<sub>2</sub>-alkyl, and NO<sub>2</sub>;

25 R<sub>4</sub> represents H, C<sub>1-2</sub> alkyl;

R<sub>5</sub> represents C(=A)NHR<sub>6</sub>, COR<sub>7</sub>, or R<sub>6</sub>;

A represents O, S, or N;

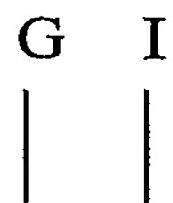
R<sub>6</sub> represents H, or C<sub>1-2</sub> alkyl;

R<sub>7</sub> represents C<sub>1-2</sub> alkyl;

L represents a linker D-E-D such that

D represents a bond or C<sub>1-4</sub> alkyl;

5



E represents C = C, CONH, NHCO, COO, N, O, S, or  $\equiv$ ; and

G and I independently represent H, C<sub>1-2</sub> alkyl; or a pharmaceutically acceptable salt thereof, provided that the compound of formula (I) is not 2-[(aminocarbonyl)amino]-

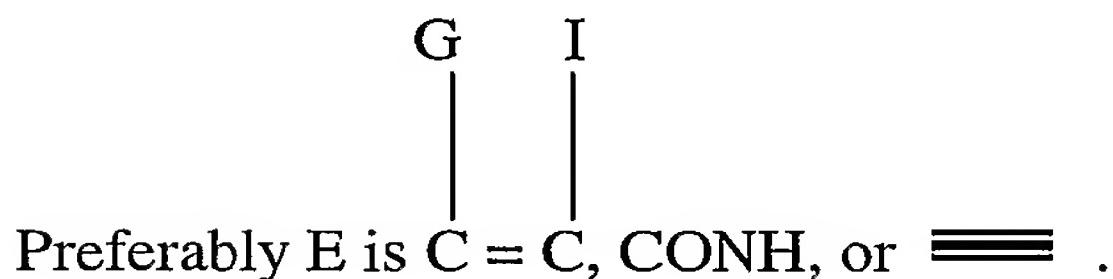
10 5-{{(4-chlorophenyl)methyl]oxyl}-3-thiophenecarboxamide.

Preferably R<sub>2</sub> is CONH<sub>2</sub>.

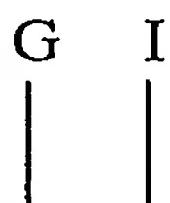
Preferably R<sub>5</sub> is C(=A)NHR<sub>6</sub>.

Preferably R<sub>6</sub> is H.

15 Preferably A is O.



20



More preferably E is C = C or  $\equiv$ .

Preferred compounds useful in the present invention include:

5-[(E)-phenyl]-ethenyl]-2-ureido-thiophene-3-carboxylic acid amide;

25 5-[(E)-2-(4-Fluoro-phenyl)- ethenyl]-2-ureido-thiophene-3-carboxylic acid amide;

5-[(E)-2-(4-Chloro-phenyl)- ethenyl]-2-ureido-thiophene-3-carboxylic acid amide;

5-Phenethyl-2-ureido-thiophene-3-carboxylic acid amide;

5-Benzyl-2-ureido-thiophene-3-carboxylic acid amide;

5-(1-Phenyl-ethyl)-2-ureido-thiophene-3-carboxylic acid amide;

30 5-Phenylethynyl-2-ureido-thiophene-3-carboxylic acid amide;

5-(4-Fluorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;

- 5-(4-Ethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
5-(4-Methoxyphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
5-(4-Chlorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide ;  
5-(4-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
5 5-(3-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
and  
5-Acetyl amino-thiophene-2,4-dicarboxylic acid 4-amide 2-[ (3-chloro-phenyl)-  
amide]; or a pharmaceutically acceptable salt thereof.

This invention provides methods for treating a variety of diseases associated  
10 with NF- $\kappa$ B activation including inflammatory and tissue repair disorders; particularly  
rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic  
obstructive pulmonary disease) osteoarthritis, osteoporosis and fibrotic diseases;  
dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-  
induced skin damage; autoimmune diseases including systemic lupus eythematosus,  
15 multiple sclerosis, psoriatic arthritis, alkylosing spondylitis, tissue and organ rejection,  
Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis,  
cancer, including Hodgkins disease, cachexia, inflammation associated with infection  
and certain viral infections, including aquired immune deficiency syndrome (AIDS),  
adult respiratory distress syndrome, and Ataxia Telangiectasia.

20

### DEFINITIONS

The present invention includes all hydrates, solvates, complexes and  
prodrugs of the compounds of this invention. Prodrugs are any covalently bonded  
compounds, which release the active parent, drug according to Formula I *in vivo*. If  
25 a chiral center or another form of an isomeric center is present in a compound of the  
present invention, all forms of such isomer or isomers, including enantiomers and  
diastereomers, are intended to be covered herein. Inventive compounds containing a  
chiral center may be used as a racemic mixture, an enantiomerically enriched  
mixture, or the racemic mixture may be separated using well-known techniques and  
30 an individual enantiomer may be used alone. In cases in which compounds have  
unsaturated carbon-carbon double bonds, both the cis (Z) and trans (E) isomers are

within the scope of this invention. In cases wherein compounds may exist in tautomeric forms, such as keto-enol tautomers, each tautomeric form is contemplated as being included within this invention whether existing in equilibrium or predominantly in one form.

5        The meaning of any substituent at any one occurrence in Formula I or any subformula thereof is independent of its meaning, or any other substituent's meaning, at any other occurrence, unless specified otherwise.

As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined by single carbon-carbon bonds and having 1-6 carbon atoms joined together.

10      The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated. Substituents on optionally substituted alkyl are selected from the group consisting of aryl, OH, O-alkyl, CO, halogen, CF<sub>3</sub>, and OCF<sub>3</sub>.

As used herein, "aryl" refers to an optionally substituted aromatic group with at least one ring having a conjugated pi-electron system, containing up to two conjugated or fused ring systems. Aryl includes carbocyclic aryl, and biaryl groups, all of which may be optionally substituted. Substituents are selected from the group consisting of halogen, C<sub>1-4</sub> alkyl, NH<sub>2</sub>, OCF<sub>3</sub>, CF<sub>3</sub>, O-alkyl, S-alkyl, CN, CHO, SO<sub>2</sub>-alkyl and NO<sub>2</sub>.

As used herein, "heteroaryl" refers to an optionally substituted aromatic group with at least one ring having a conjugated pi-electron system, containing up to two conjugated or fused ring systems and 1-3 heteroatoms selected from O, S and N. Heteroaryl includes carbocyclic heteroarylaryl, aryl-heteroaryl and biheteroarylaryl groups, all of which may be optionally substituted. Preferred aryl include phenyl and naphthyl. More preferred aryl include phenyl. Preferred substituents are selected from the group consisting of halogen, C<sub>1-4</sub> alkyl, NH<sub>2</sub>, OCF<sub>3</sub>, CF<sub>3</sub>, O-alkyl, S-alkyl, CN, CHO, SO<sub>2</sub>-alkyl and NO<sub>2</sub>. Examples of heteroaryl rings included pyrrole, furan, thiophene, indole, isoindole, benzofuran, isobenzofuran, benzothiphene, pyridine, quinoline, isoquinoline, quinolizine, pyrazole, imidazole, isoxazole, oxazole, isothiazole, thiazole, pyridazine, pyrimidine, and pyrazine.

30      As used herein "halogen" refers to include F, Cl, Br, and I.

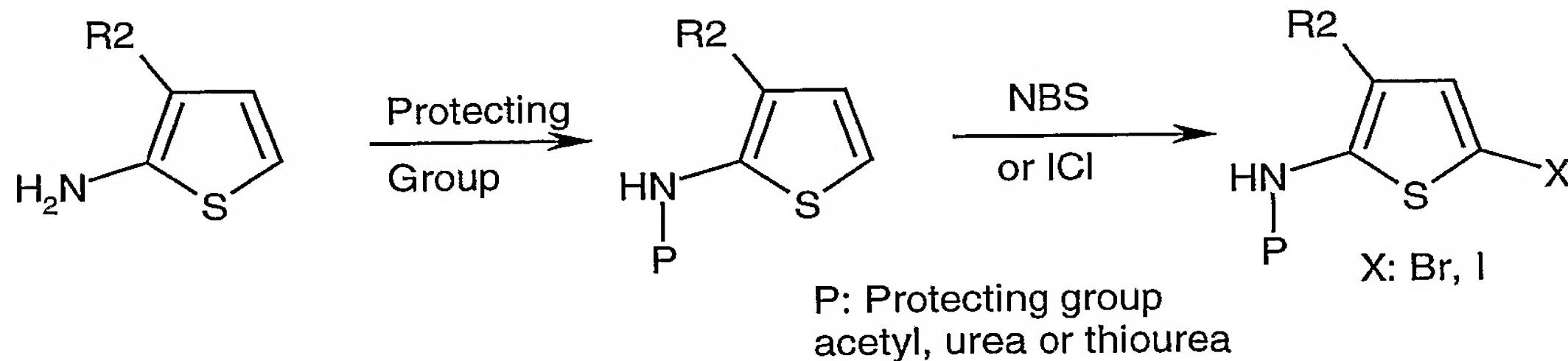
### METHODS OF PREPARATION

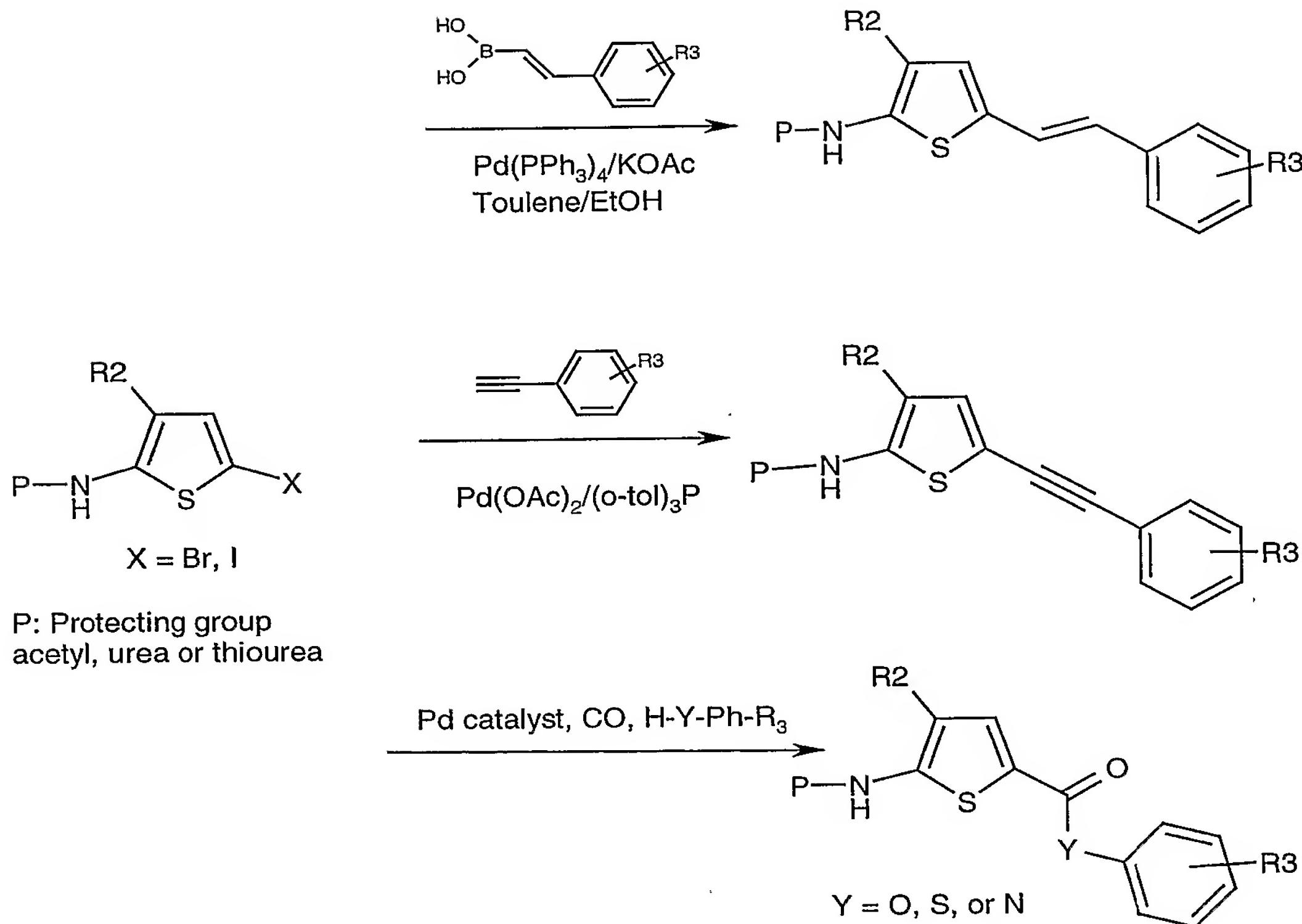
The following methods and examples are intended to be illustrative of the present invention but not limiting in any way.

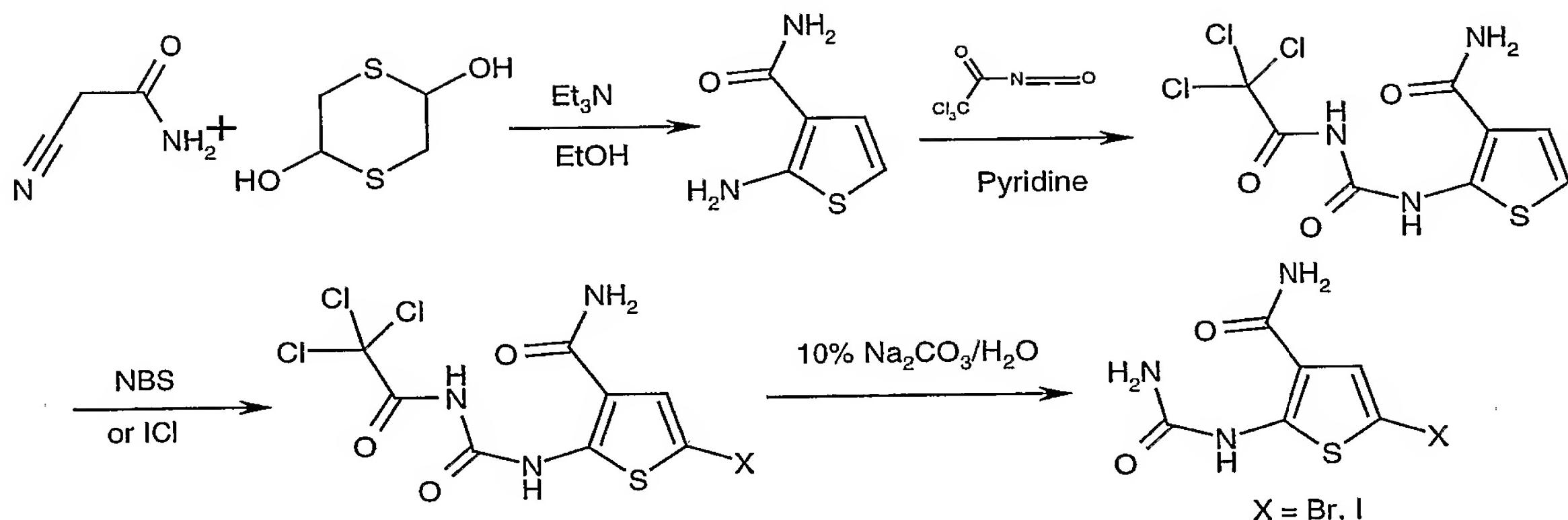
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- The general preparation of compounds described in Formula 1 is shown in Schemes 1, 2, 3, and 4. The key 5-bromo- or 5-iodo-2-aminothiophene intermediate was prepared from the corresponding protected 2-aminothiophene by treatment with N-bromosuccinimide (NBS) or iodochloride (ICl), respectively (Scheme 1).
- 10 Utilizing the 5-bromo- or 5-iodo-2-aminothiophene, analogs with olefin linker were prepared via Suzuki coupling; analogs with an acetylene linker at 5-position were prepared via Sonogashira coupling; analogs with amide, ester, thioester linker at 5-position were prepared via palladium catalyzed carbonylation (Scheme 2).
- 15 Alternately, 2-aminothiophene 3-carboxylic amides can be prepared by reacting 2-cyanoacetamide with [1,4]-dithiane-2, 5-diol followed by the protection with trichloroacetyl isocyanate. The protected thiophene is then halogenated by N-bromosuccinimide or iodochloride. Deprotection in sodium carbonate aqueous solution furnished the 5-bromo or 5-iodo-2-ureidothiophene-3-carboxylic amide (Scheme 3). Aminothiophene with alkyl linkers at 5-position can be prepared by reacting the required aldehydes with 2-cyano acetamide followed by the reaction with chlorosulfonyl isocynate to give the 2-ureido-3-amidethiophenes with alkyl linker at 5-position (Scheme 4).
- 20

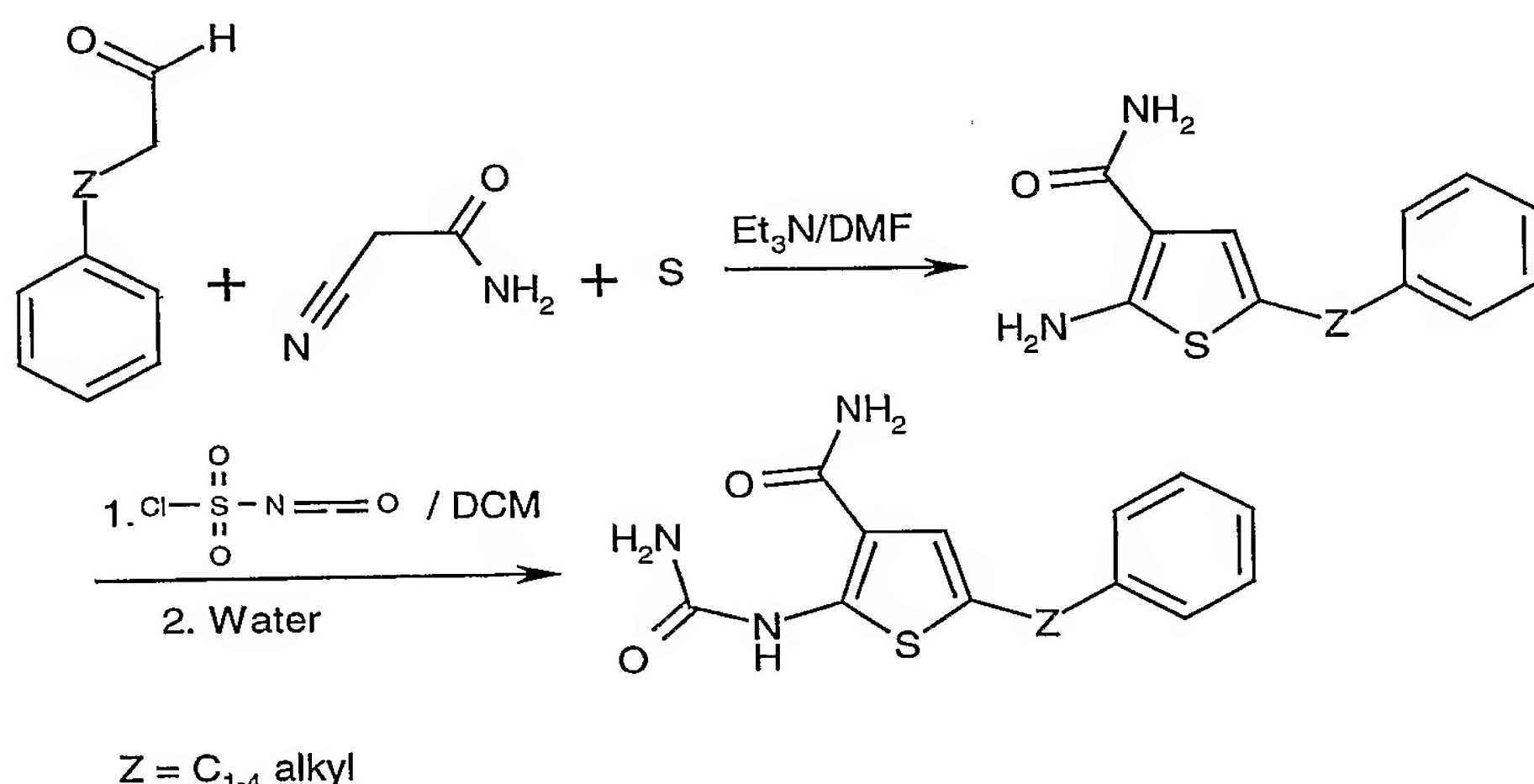
**Scheme 1**



**Scheme 2**

**Scheme 3**

5

**Scheme 4****General Procedures**

Nuclear magnetic resonance spectra were recorded at 400 MHz using on a  
 10 Bruker AC 400 spectrometer. CDCl<sub>3</sub> is deuteriochloroform, DMSO-d<sub>6</sub> is hexadeuteriodimethylsulfoxide, and CD<sub>3</sub>OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (d) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz. Mass spectra were collected on a LCMS system using a Sciex

15

API 150EX instrument [1 x 40 mm Aquasil (C18) column, gradient 4.5%-90% acetonitrile-water (0.02% TFA) over 3.2 min, detection by mass, UV at 214 nm and by evaporative light-scattering. Mass was determined using electrospray ionization techniques.. All temperatures are reported in degree Celsius. Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Both flash and gravity chromatography were carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel. Preparative HPLC were performed using a Gilson Preparative System using a 50 x 20 mml S-5μl C18 reverse phase column eluting with a 10-80 gradient (0.1%TFA in acetonitrile/0.1% aqueous TFA).  
5  
10

The following examples are intended to be illustrative of the present invention but not limiting in any way.

### Example 1

15 **Preparation 5-Bromo-2-Ureido-thiophene-3-carboxylic acid amide**

**a). 2-Amino-thiophene-3-carboxylic acid amide**

Triethylamine (36 mL, 256 mmol) was added to the solution of 2-cyanoacetamide (10.8 g, 128 mmol) and [1,4]-dithiane-2,5-diol (19.5 g, 128 mmol)  
20 in ethanol (400 mL). The resulting reaction mixture was stirred at room temperature for 5 min, then heated at reflux for 2 h. After the reaction mixture is cooled to room temperature, it was filtered and concentrated under vacuum. The residue was then taken up into ethyl acetate (400 mL) and washed with aqueous sodium hydroxide (1 M, 1x200 mL), water (2x200mL) and brine (1x200 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum to give the above titled compound as a light yellow solid (16 g, 113 mmol, 88% yield). LC-MS  
25 [M+H]<sup>+</sup> m/z 143.

**b) 2-[3-(2,2,2-Trichloro-ethanoyl)-ureido]-thiophene-3-carboxylic acid amide**

30 Trichloroacetyl isocyanate (10 g, 53 mmol) was added dropwise to the solution of 2-amino-thiophene-3-carboxylic acid amide (3.8g, 26.5 mmol) in

pyridine (110 mL) at 0°C. The resulting reaction mixture was stirred at 0°C. The temperature of the reaction mixture was raised to room temperature over a 3 h period until LC-MS showed no remaining starting material. Diethyl ether (100 mL) was added to the reaction mixture and the resulting precipitate was filtered and dried under vacuum to give the above titled compound as a light grey solid (8.7 g, 26.3 mmol, 99 % yield).

**c) 5-Bromo-2-[3-(2,2,2-trichloro-ethanoyl)-ureido]-thiophene-3-carboxylic acid amide**

N-Bromosuccinimide (1.56 g, 8.8 mmol) was added to a solution of 2-[3-(2,2,2-trichloro-ethanoyl)-ureido]-thiophene-3-carboxylic acid amide (2.9 g, 8.8 mmol) in acetic acid (30 mL) and chloroform (10 mL) at room temperature. The resulting solution was stirred under nitrogen with an aliquot was taken for LC-MS every 10 min. After 30 min., the LC-MS showed no remaining starting material. Diethyl ether (80 mL) was added to the solution and the resulting precipitate was filtered and dried under vacuum to give the above titled compound as a light brown solid (3.5 g, 8.55 mmol, 97 % yield).

**d) 5-Bromo-2-ureido-thiophene-3-carboxylic acid amide**

Sodium carbonate solution (10% aqueous, 30 mL) was added to a solution of 5-bromo-2-[3-(2,2,2-trichloro-ethanoyl)-ureido]-thiophene-3-carboxylic acid amide (1.0 g, 2.44 mmol) in EtOH (10 mL). The resulting reaction mixture was stirred under nitrogen overnight. After one third of the solvent was removed under vacuum, the reaction mixture was filtered. The precipitate was washed with water (3 x 50 mL) and dried in vacuum to give the above titled compound as a light brown solid (510 mg, 2.16 mmol, 89 % yield).

**Example 2**

**Preparation of 5-[(E)-Phenyl]-ethenyl]-2-ureido-thiophene-3-carboxylic acid amide 5**

A solution of 5-bromo-2-ureido-thiophene-3-carboxylic acid amide (0.066 g, 0.25 mmol) in absolute ethanol (2 mL) and toluene (2 mL) in a 5 mL reaction vial was treated with potassium acetate (0.05 g, 0.5 mmol), tetrakis (triphenylphosphine) palladium (0.015 g, 5 mole%), and trans-2-phenylethenylboronic acid (0.041 g, 0.28 mmol). The reaction vial was then sealed and heat at 90-95 °C for 12 h. The solvent was removed under vacuum and the residue was taken up into DMSO (5 mL), filtered and purified utilizing a Gilson preparative HPLC system to give the above titled product as a light brown solid (29 mg, 0.1 mmol, 40 % yield). LC-MS [M+H]<sup>+</sup> m/z 288.

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**Example 3****Preparation of 5-[(E)-2-(4-Fluoro-phenyl)-ethenyl]-2-ureido-thiophene-3-carboxylic acid amide**

5-[(E)-2-(4-Fluoro-phenyl)-ethenyl]-2-ureido-thiophene-3-carboxylic acid amide was prepared by the same procedure as described in Example 2 except that trans-2-phenylethenylboronic acid was replaced with trans-2-(4-fluorophenyl)vinylboronic acid. The above titled compound was isolated as a light brown solid (31 mg, 0.1 mmol, 40 % yield). ESMS  $[M+H]^+$  m/z 306.

10

**Example 4**

**Preparation of 5-[(E)-2-(4-Chloro-phenyl)-ethenyl]-2-ureido-thiophene-3-carboxylic acid amide** 5-[(E)-2-(4-Chloro-phenyl)-ethenyl]-2-ureido-thiophene-3-carboxylic acid amide was prepared using the same procedure as described in Example 2 except that trans-2-phenylethenylboronic acid was replaced with trans-2-(4-chlorophenyl)ethenylboronic acid. The above titled compound was isolated as a light brown solid (28 mg, 0.087 mmol, 35% yield). ESMS  $[M+H]^+$  m/z 322.

15  
**Example 5****Preparation of 5-Phenethyl-2-ureido-thiophene-3-carboxylic acid amide**

20 Palladium on carbon (10%, 0.01 g) was added to a solution of 5-[(E)-styryl]-vinyl]-2-ureido-thiophene-3-carboxylic acid amide (0.02 g, 0.06 mmol) in acetic acid (1 mL) and ethanol (1 mL) and the resulting reaction mixture was placed into a Parr reactor and treated with 20 psi H<sub>2</sub> for 72 h. The reaction mixture was then filtered through Celite and purified by flash chromatography (silica gel, 100 % ethyl acetate) 25 to give the above titled compound as a white solid (5 mg, 25 % yield). ESMS  $[M+H]^+$  m/z 290.

**Example 6****Preparation of 5-Benzyl-2-ureido-thiophene-3-carboxylic acid amide****a) 2-Amino-5-benzyl-thiophene-3-carboxylic acid amide trifluoroacetate**

Triethyl amine (0.28 mL, 2.0 mmol) was added dropwise to a mixture of 2-cyano-acetamide (0.168 g, 2.0 mmol), sulfur (0.064 g, 2.0 mmol) and 3-phenylpropionaldehyde (0.29 g, 2.0 mmol) in DMF (8 mL) and resulting reaction mixture was stirred at room temperature overnight. The reaction mixture was then filtered and purified utilizing a Gilson preparative HPLC to give the above titled compound as light yellow solid (0.25 g, 1.06 mmol, 53 % yield). LC-MS  $[M+H]^+$  m/z 233.

10

**b) 5-Benzyl-2-ureido-thiophene-3-carboxylic acid amide**

Chlorosulfonyl isocyanate (0.09 mL, 1.03 mmol) was added dropwise under nitrogen to a solution of 2-amino-5-benzyl-thiophene-3-carboxylic acid amide (0.2 g, 0.86 mmol) in dry dichloromethane (9 mL) at 0 °C and. The temperature of the resulting stirred reaction mixture was slowly raised from 0 °C to room temperature over 1 h. Once the LC-MS showed no starting material remaining, the reaction was quenched with water (1 mL) and stirred for 10 additional min. The solvent was removed under vacuum and the residue was taken up into DMSO (2 mL) and purified utilizing a Gilson preparative HPLC to give the above titled compound as a light brown solid (0.16 g, 67 %). LC-MS  $[M+H]^+$  m/z 276.

**Example 7****Preparation of 5-(1-Phenyl-ethyl)-2-ureido-thiophene-3-carboxylic acid amide**

25   **a) 2-Amino-5-(1-phenyl-ethyl)-thiophene-3-carboxylic acid amide trifluoroacetate**

2-Amino-5-(1-phenyl-ethyl)-thiophene-3-carboxylic acid amide trifluoroacetate was prepared by the same procedure as described in Example 6a except that 3-phenylpropionaldehyde was replaced with 3-phenylbutyraldehyde to give the above titled compound as a light brown solid (17 % yield). LC-MS  $[M+H]^+$  m/z 247.

**b) 5-(1-Phenyl-ethyl)-2-ureido-thiophene-3-carboxylic acid amide**

5-(1-Phenyl-ethyl)-2-ureido-thiophene-3-carboxylic acid amide was prepared by the same procedure as Example 6b except that 2-amino-5-benzyl-thiophene-3-carboxylic acid amide was replaced with 2-amino-5-(1-phenyl-ethyl)-thiophene-3-carboxylic acid amide to give the above titled compound as a light brown solid (87 % yield). LC-MS  $[M+H]^+$  m/z 290.

**Example 8****10 Preparation of 5-Phenylethynyl-2-ureido-thiophene-3-carboxylic acid amide**

5-Bromo-2-ureido-thiophene-3-carboxylic acid amide (0.264 g, 1.0 mmol), copper (I) iodide (0.005 g), triphenyl phosphine (0.030 g, 15mole%), palladium acetate (0.010 g, 5% mole%), triethyl amine (1 mL) and phenylacetylene (0.1 g, 1.1 mmol) in DMF (4 mL) was added to a 9 mL reaction vial. The reaction vial was sealed and heated to 100 °C in a Smith Microwave Synthesizer for 20 min. Water (5 mL) and ethyl acetate (20 mL) were added to the reaction mixture, the organic phase was separated and then washed with brine (2x 10 mL) and water (1 x10 mL), and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed under vacuum and the crude residue was taken into DMSO (5 mL) and purified utilizing a Gilson preparative HPLC to give the above titled compound as a light brown solid (0.050 g, 0.18 mmol, 18 % yield). LC-MS  $[M+H]^+$  m/z 286.

**Example 9****25 Preparation of 5-(4-Fluorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide**

5-(4-Fluorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide was prepared by the same procedure as Example 8 except phenylacetylene was replaced with 4-fluorophenylacetylene to give the above titled compound as a light brown solid. LC-MS  $[M+H]^+$  m/z 304.

30

**Example 10**

**Preparation of 5-(4-Ethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide**

5-(4-Ethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide was prepared by the same procedure as Example 8 except phenylacetylene was replaced with 4-ethylphenylacetylene to give the above titled compound as a light brown solid. LC-MS  $[M+H]^+$  m/z 314.

**Example 11**

**Preparation of 5-(4-Methoxyphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide**

5-(4-Methoxyphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide was prepared by the same procedure as Example 8 except phenylacetylene was replaced with 4-methoxyphenylacetylene to give the above titled compound as a light brown solid. LC-MS  $[M+H]^+$  m/z 316.

15

**Example 12**

**Preparation of 5-(4-Chlorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide**

5-(4-Chlorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide was prepared by the same procedure as Example 8 except phenylacetylene was replaced with 4-chlorophenylacetylene to give the above titled compound as a light brown solid. LC-MS  $[M+H]^+$  m/z 320.

**Example 13**

25 **Preparation of 5-(4-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide**

5-(4-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid was prepared by the same procedure as Example 8 except phenylacetylene was replaced with 4-trifluoromethylphenylacetylene to give the above titled compound 30 as a light brown solid. LC-MS  $[M+H]^+$  m/z 354.

**Example 14****Preparation of 5-(3-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide**

5-*(3-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide* was prepared by the same procedure as Example 8 except phenylacetylene was replaced with 3-trifluoromethylphenylacetylene to give the above titled compound as a light brown solid. LC-MS  $[M+H]^+$  m/z 354.

**Example 15****10 Preparation of 2-Acetylamino-5-bromo-thiophene-3-carboxylic acid amide****a). 2-Acetylamino-thiophene-3-carboxylic acid amide**

Acetyl chloride (4.2 mL, 59 mmol) was added dropwise to a solution of 2-amino-thiophene-3-carboxylic acid amide (7.7 g, 54.2 mmol) in pyridine (100 mL) at 0°C. The temperature of the reaction mixture was raised to room temperature over a 3 h period until no remaining starting material was present based on LC-MS . The solvent was then removed under vacuum to give the above titled compound as brown solid (9.7 g, 52.7 mmol, 97.2% yield). LCMS  $[M+H]^+$  m/z 185.

20

**b). 2-Acetylamino-5-bromo-thiophene-3-carboxylic acid amide**

2-Acetylamino-thiophene-3-carboxylic acid amide (3.89 g, 27.4 mmol) was suspended in the aqueous solution of sodium acetate (2.47 g, 30.1 mmol)(136 mL). Bromine (1.4 mL, 27.4 mmol) was added dropwise to the solution. The resulting solution was stirred under nitrogen overnight. Once the LC-MS showed no remaining starting materials, the reaction mixture was then filtered and washed with water (50 mL). The filtrate was then purified utilizing a Gilson preparative HPLC to give the above titled compound as a brown solid (1 g, 13.8%). LC-MS  $[M+H]^+$  m/z 263.

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**Example 16**

**Preparation of 5-Acetylamino-thiophene-2,4-dicarboxylic acid 4-amide-2-[(3-chloro-phenyl)-amide]**

2-Acetylamino-5-bromo-thiophene-3-carboxylic acid amide (0.1 g, 0.38 mmol), *bis*-triphenyl phosphene palladium(II) chloride (0.013 g, 5 mole%), 5 diisopropylethylamine (0.16 g, 1.2 mmol), water (0.015 mL, 0.87 mmol), 3-chloroaniline (0.12 g, 0.78 mmol) in NMP (4 mL) was added to a 9 mL reaction vial. Carbon monoxide was bubbled through the reaction mixture via a plastic balloon filled with carbon monoxide gas. The reaction vial was sealed and stirred at 90 °C for 12 h. Water (5 mL) and ethyl acetate (20 mL) were added to the reaction 10 mixture, the organic phase was separated and then washed with brine (2x 10 mL) and water (1 x10 mL), and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed under vacuum and the crude residue was taken into DMSO (5 mL) and purified utilizing a Gilson preparative HPLC to give the above titled product as a light brown solid (0.020 g, 0.17 mmol, 16 % yield). LC-MS [M+H]<sup>+</sup> 15 m/z 338.

This invention provides a pharmaceutical composition, which comprises a compound according to Formula I and a pharmaceutically acceptable carrier, diluent or excipient. Accordingly, the compounds of Formula I may be used in the 20 manufacture of a medicament. Pharmaceutical compositions of the compounds of Formula I prepared as hereinbefore described may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation may be a buffered, isotonic, aqueous solution. 25 Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water or buffered sodium or ammonium acetate solution. Such formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer for insufflation. It may be desirable to add excipients such as polyvinylpyrrolidone, 30 gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride or sodium citrate.

Alternately, these compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. Liquid carriers include syrup, peanut oil, olive oil, saline and water. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 g per dosage unit. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

Typical compositions for inhalation are in the form of a dry powder, solution, suspension or emulsion. Administration may for example be by dry powder inhaler (such as unit dose or multi-dose inhaler, e.g., as described in US Patent 5590645 or by nebulisation or in the form of a pressurized aerosol. Dry powder compositions typically employ a carrier such as lactose, trehalose or starch. Compositions for nebulisation typically employ water as vehicle. Pressurized aerosols typically employ a propellant such as dichlorodifluoromethane, trichlorofluoromethane or, more preferably, 1,1,1,2-tetrafluoroethane, 1,1,1,2,3,3-heptafluoro-n-propane or mixtures thereof. Pressurized aerosol formulations may be in the form of a solution (perhaps employing a solubilising agent such as ethanol) or a suspension which may be excipient free or employ excipients including surfactants and/or co-solvents (e.g. ethanol). In dry powder compositions and suspension aerosol compositions the active ingredient will preferably be of a size suitable for inhalation (typically having mass median diameter (MMD) less than 20 microns, e.g., 1-10 especially 1-5 microns). Size reduction of the active ingredient may be necessary, e.g., by micronisation.

Pressurized aerosol compositions will generally be filled into canisters fitted with a valve, especially a metering valve. Canisters may optionally be coated with a plastics material e.g. a fluorocarbon polymer as described in WO96/32150. Canisters will be fitted into an actuator adapted for buccal delivery.

5       Typical compositions for nasal delivery include those mentioned above for inhalation and further include non-pressurized compositions in the form of a solution or suspension in an inert vehicle such as water optionally in combination with conventional excipients such as buffers, anti-microbials, tonicity modifying agents and viscosity modifying agents which may be administered by nasal pump.

10      For rectal administration, the compounds of this invention may also be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and molded into a suppository.

15      The methods of the present invention include topical inhaled and intracolonic administration of the compounds of Formula I. By topical administration is meant non-systemic administration, including the application of a compound of the invention externally to the epidermis, to the buccal cavity and instillation of such a compound into the ear, eye and nose, wherein the compound does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration. The amount of a compound of the  
20      invention (hereinafter referred to as the active ingredient) required for therapeutic or prophylactic effect upon topical administration will, of course, vary with the compound chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician

25      While it is possible for an active ingredient to be administered alone as the raw chemical, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.01 to 5.0 wt% of the formulation.

30      The topical formulations of the present invention, both for veterinary and for human medical use, comprise an active ingredient together with one or more acceptable carriers therefor and optionally any other therapeutic ingredients. The

carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required such as: liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container, which is then sealed and sterilized by autoclaving, or maintaining at 90-100 °C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic

soap, a mucilage, an oil of natural origin such as almond, corn, arachis, castor or olive oil, wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active agent such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or in organic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

10

### UTILITY OF THE PRESENT INVENTION

The compounds of Formula I are useful as inhibitors of the IKK-beta kinase phosphorylation of I $\kappa$ B and as such are inhibitors of NF- $\kappa$ B activation. The present method utilizes compositions and formulations of said compounds, including pharmaceutical compositions and formulations of said compounds.

The present invention particularly provides methods of treatment of diseases associated with inappropriate NF- $\kappa$ B activation, which methods comprise administering to an animal, particularly a mammal, most particularly a human in need thereof one or more compounds of Formula I. The present invention particularly provides methods for treating inflammatory and tissue repair disorders, particularly rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease); osteoarthritis, osteoporosis and fibrotic diseases; dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage, autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkins disease, cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome and Ataxia.

30 Telangiectasia.

For acute therapy, parenteral administration of one or more compounds of Formula I is useful. An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although an intramuscular bolus injection is also useful. Typically, the 5 parenteral dose will be about 0.01 to about 50 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the concentration of drug in the plasma at a concentration effective to inhibit IKK-beta and therefore activation of NF- $\kappa$ B. The compounds are administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 80 mg/kg/day. The precise amount of a compound used 10 in the present method which is therapeutically effective, and the route by which such compound is best administered, is readily determined by one of ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect.

The compounds of Formula I may also be administered orally to the patient, 15 in a manner such that the concentration of drug is sufficient to inhibit IKK-beta and therefore activation of NF- $\kappa$ B or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 20 0.5 to about 20 mg/kg.

The compounds of Formula I may also be administered topically to the patient, in a manner such that the concentration of drug is sufficient to inhibit IKK-beta and therefore activation of NF- $\kappa$ B or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the 25 compound is administered in a topical formulation of between about 0.01% to about 5% w/w.

No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The ability of the compounds described herein to inhibit the activation of 30 NF- $\kappa$ B is clearly evidenced in their ability to inhibit the phosphorylation of the N-terminal fragment of I $\kappa$ B- $\alpha$  by IKK- $\beta$ . These compounds also block the degradation

of I $\kappa$ B- $\alpha$  and the nuclear translocation of NF- $\kappa$ B in human monocytes and other mammalian cells upon activation of the cells with a pro-inflammatory stimuli (e.g., TNF- $\alpha$ , LPS, etc.). In addition these compounds inhibit pro-inflammatory mediator production from LPS-stimulated human monocytes and stimulated human primary synovial fibroblasts. The utility of the present NF- $\kappa$ B inhibitors in the therapy of diseases is premised on the importance of NF- $\kappa$ B activation in a variety of diseases.

NF- $\kappa$ B plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as TNF, IL-1 $\beta$ , IL-6 and IL-8 (Mukaida *et al.*, 1990; Liberman and Baltimore, 1990; Matsusaka *et al.*, 1993), cell adhesion molecules, such as ICAM and VCAM (Marui *et al.*, 1993; Kawai *et al.*, 1995; Ledebur and Parks, 1995), and inducible nitric oxide synthase (iNOS) (Xie *et al.*, 1994; Adcock *et al.*, 1994). Such mediators are known to play a role in the recruitment of leukocytes at sites of inflammation and in the case of iNOS, may lead to organ destruction in some inflammatory and autoimmune diseases (McCartney-Francis *et al.*, 1993; Kleemann *et al.*, 1993).

Evidence for an important role of NF- $\kappa$ B in inflammatory disorders is obtained in studies of asthmatic patients. Bronchial biopsies taken from mild atopic asthmatics show significant increases in the number of cells in the submucosa staining for activated NF- $\kappa$ B, total NF- $\kappa$ B, and NF- $\kappa$ B-regulated cytokines such as GM-CSF and TNF $\alpha$  compared to biopsies from normal non-atopic controls (Wilson *et al.*, 1998). Furthermore, the percentage of vessels expressing NF- $\kappa$ B immunoreactivity is increased as is IL-8 immunoreactivity in the epithelium of the biopsy specimens (Wilson *et al.*, 1998). As such, inhibition of IL-8 production through the inhibition of NF- $\kappa$ B, as has been demonstrated by these compounds would be predicted be beneficial in airway inflammation.

Recent studies suggest that NF- $\kappa$ B may also play a critical role in the pathogenesis of inflammatory bowel disease (IBD). Activated NF- $\kappa$ B is seen in colonic biopsy specimens from Chron's disease and ulcerative colitis patients (Ardite *et al.*, 1998; Rogler *et al.*, 1998; Schreiber *et al.*, 1998). Activation is evident in the inflamed mucosa but not in uninflamed mucosa (Ardite *et al.*, 1998; Rogler *et al.*,

1998) and is associated with increased IL-8 mRNA expression in the same sites (Ardite *et al.*, 1998). Furthermore, corticosteroid treatment strongly inhibits intestinal NF-κB activation and reduces colonic inflammation (Ardite *et al.*, 1998; Schreiber *et al.*, 1998). Again, inhibition of IL-8 production through the inhibition of NF-κB, as has been demonstrated by these compounds would be predicted be beneficial in inflammatory bowel disease.

Animal models of gastrointestinal inflammation provide further support for NF-κB as a key regulator of colonic inflammation. Increased NF-κB activity is observed in the lamina propria macrophages in 2,4,6,-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice with p65 being a major component of the activated complexes (Neurath *et al.*, 1996; Neurath and Pettersson, 1997). Local administration of p65 antisense abrogates the signs of established colitis in the treated animals with no signs of toxicity (Neurath *et al.*, 1996; Neurath and Pettersson, 1997). As such, one would predict that small molecule inhibitors of NF-κB would be useful in the treatment of IBD.

Further evidence for a role of NF-κB in inflammatory disorders comes from studies of rheumatoid synovium. Although NF-κB is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF-κB is present in the nuclei, and hence active, in the cells comprising human rheumatoid synovium (Handel *et al.*, 1995; Marok *et al.*, 1996; Sioud *et al.*, 1998) and in animal models of the disease (Tsao *et al.*, 1997). The staining is associated with type A synoviocytes and vascular endothelium (Marok *et al.*, 1996). Furthermore, constitutive activation of NF-κB is seen in cultured synoviocytes (Roshak *et al.*, 1996; Miyazawa *et al.*, 1998) and in synovial cell cultures stimulated with IL-1 $\beta$  or TNF $\alpha$  (Roshak *et al.*, 1996; Fujisawa *et al.*, 1996; Roshak *et al.*, 1997). Thus, the activation of NF-κB may underlie the increased cytokine production and leukocyte infiltration characteristic of inflamed synovium. The ability of these compounds to inhibit NF-κB and thereby inhibit the production of pro-inflammatory mediators (e.g., cytokines and prostanooids) by these cells would be predicted to yield benefit in rheumatoid arthritis.

### Biological Assays

The compounds of this invention may be tested in one of several biological assays to determine the concentration of compound, which is required to have a  
5 given pharmacological effect.

NF- $\kappa$ B activity may also be measured in an electrophoretic mobility shift assay (EMSA) to assess the presence of NF- $\kappa$ B protein in the nucleus. The cells of interest are cultured to a density of  $1 \times 10^6$  /mL. The cells are harvested by centrifugation, washed in PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and resuspended in PBS with  
10  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  at  $1 \times 10^7$  cells/mL. To examine the effect of compound on the activation of NF- $\kappa$ B, the cell suspensions are treated with various concentrations of drug or vehicle (DMSO, 0.1%) for 30 min. at 37 °C prior to stimulation with TNF- $\alpha$  (5.0 ng/mL) for an additional 15 min. Cellular and nuclear extracts are prepared follows. Briefly, at the end of the incubation period the cells ( $1 \times 10^7$  cells) are  
15 washed 2x in PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . The resulting cell pellets are resuspended in 20 uL of Buffer A (10 mM Hepes (pH 7.9), 10 mM KCl, 1.5 mM  $\text{MgCl}_2$ , 0.5 mM dithiothreitol (DTT) and 0.1% NP-40) and incubated on ice for 10 min. The nuclei are pelleted by microcentrifugation at 3500 rpm for 10 min at 4 °C. The resulting supernatant was collected as the cellular extract and the nuclear pellet was  
20 resuspended in 15 uL Buffer C (20 mM Hepes (pH 7.9), 0.42 M NaCl, 1.5mM  $\text{MgCl}_2$ , 25% glycerol, 0.2 mM EDTA, 0.5 mM DTT, and 0.5 mM phenylmethylsulphonyl fluoride (PMSF)). The suspensions are mixed gently for 20 min at 4 °C then microcentrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant is collected and diluted to 60 uL with Buffer D (20mM Hepes (pH 7.9),  
25 50 mM KCl, 20% glycerol, 0.2 mM EDTA, 0.5 mM DTT, and 0.5 mM PMSF). All samples are stored at –80 °C until analyzed. The protein concentration of the extracts is determined according to the method of Bradford (Bradford, 1976) with BioRad reagents.

The effect of compounds on transcription factor activation is assessed in an  
30 electrophoretic mobility shift assay (EMSA) using nuclear extracts from treated cells as described above. The double stranded NF- $\kappa$ B consensus oligonucleotides (5'-

AGTTGAGGGGACTTCCCAGGC-3') are labelled with T<sub>4</sub> polynucleotide kinase and [ $\text{g-}^{32}\text{P}$ ]ATP. The binding mixture (25 uL) contains 10 mM Hepes-NaOH (pH 7.9), 4 mM Tris-HCl (pH 7.9), 60 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 0.3 mg/mL bovine serum albumin, and 1 ug poly(dI-dC)•poly(dI-dC). The 5 binding mixtures (10 ug nuclear extract protein) are incubated for 20 min at room temperature with 0.5 ng of  $^{32}\text{P}$ -labelled oligonucleotide (50,000-100,000 cpm) in the presence or absence of unlabeled competitor after which the mixture is loaded on a 4% polyacrylamide gel prepared in 1X Tris borate/EDTA and electrophoresed at 200 V for 2 h. Following electrophoresis the gels are dried and exposed to film for 10 detection of the binding reaction.

The effect of compounds on the phosphorylation of I $\kappa$ B may be monitored in a Western blot. Cellular extracts are subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels (BioRad, Hercules, CA) and the proteins transferred to nitrocellulose sheets (Hybond<sup>tm</sup>-ECL, Amersham 15 Corp., Arlington Heights, IL). Immunoblot assays are performed using a polyclonal rabbit antibody directed against I $\kappa$ B $\alpha$  or I $\kappa$ B $\beta$  followed with a peroxidase-conjugated donkey anti-rabbit secondary antibody (Amersham Corp., Arlington Heights, IL). Immunoreactive bands are detected using the Enhanced Chemiluminescence (ECL) assay system (Amersham Corp., Arlington Heights, IL).

20 Assays for I $\kappa$ B kinases were conducted as follows: IKK- $\alpha$  was expressed as a hexa-histidine tagged protein in baculovirus-infected insect cells and purified over a Ni-NTA affinity column. Kinase activity was assayed using 50 ng of purified protein in assay buffer (20 mM Hepes, pH 7.7, 2 mM MgCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, 10 mM  $\beta$ -glycerophosphate, 10 mM NaF, 10 mM PNPP, 0.3 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM benzamidine, 2  $\mu$ M PMSF, 10  $\mu$ g/ml aprotinin, 1 ug/mL leupeptin, 1 ug/mL pepstatin, 1mM DTT) containing various concentrations of compound or DMSO vehicle and ATP as indicated (Pharmacia Biotech Inc., Piscataway, NJ). The 25 reaction was started by the addition of 200 ng I $\kappa$ B-GST (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), in a total volume of 50 uL. The reaction was allowed to react 30 proceed for 1 h. at 30 °C after which the reaction was terminated by the addition of

EDTA to a final concentration of 20 mM. Kinase activity was determined by dissociation-enhanced lanthanide fluorescence immunoassay (Wallac Oy, Turku, Finland) using a phospho-I $\kappa$ B- $\alpha$  (Ser32) antibody (New England Biolabs, Inc., Beverly, MA) and an Eu<sup>3+</sup>-labelled anti-rabbit IgG (Wallac Oy, Turku, Finland).

5 The plates were read in a VICTOR 1420 Multilabel Counter (Wallac), using a standard europium protocol (excitation 340 nm, emission 615 nm; fluorescence measured for 400  $\mu$ s after a 400 usec delay). Data are expressed as fluorescence (cps) units.

IKK- $\beta$  was expressed as a GST-tagged protein, and its activity was assessed  
10 in a 96-well scintillation proximity assay (SPA). Briefly, IKK- $\beta$  was diluted in assay buffer as described above (20 nM final), with various concentrations of compound or DMSO vehicle, 240 nM ATP and 200 nCi [ $\gamma$ -<sup>33</sup>P]-ATP (10 mCi/mL, 2000 Ci/mmol; NEN Life Science Products, Boston, MA). The reaction was started with the addition of a biotinylated peptide comprising amino acids 15 – 46 of I $\kappa$ B- $\alpha$   
15 (American Peptide) to a final concentration of 2.4  $\mu$ M, in a total volume of 50  $\mu$ L. The sample incubated for one hour at 30 °C, followed by the addition of 150  $\mu$ L of stop buffer (PBS w/o Ca<sup>2+</sup>, Mg<sup>2+</sup>, 0.1% Triton X-100 (v/v), 10 mM EDTA)  
20 containing 0.2 mg streptavidin-coated SPA PVT beads (Amersham Pharmacia Biotech, Piscataway, NJ). The sample was mixed, incubated for 10 min. at room temperature, centrifuged (1000 xg, 2 minutes), and measured on a Hewlett-Packard TopCount.

In addition, IKK- $\beta$  or IKK- $\alpha$  activity is measured by phosphorylation of recombinant GST-I $\kappa$ appaBalpa using time-resolved fluorescence resonance energy transfer (TR-FRET) in 384-well microtitre plates. Briefly IKK- $\beta$  or IKK- $\alpha$  is diluted  
25 in assay buffer (50 mM HEPES pH 7.4 containing 10 mM magnesium chloride, 1 mM CHAPS, 1 mM DTT and 0.01% w/v BSA) to 5 nM final concentration. This is added to various concentrations of compound or DMSO vehicle and the reaction started by addition of 25 nM GST-I $\kappa$ appaBalpa and 1  $\mu$ M ATP in assay buffer to a volume of 30  $\mu$ L. After incubation for 30 min at ambient temperature the reaction  
30 was stopped by addition of 50 mM pH 7.4 EDTA (15  $\mu$ L). Detection of

phosphorylated product was achieved by addition of a LANCE europium chelate labelled specific anti-phosphoserine monoclonal antibody at 0.5 nM final concentration (Cell signalling Technology via Perkin Elmer) and allophycocyanin labelled anti-GST antibody at 10 nM final concentration (Prozyme) to give a final 5 volume of 60 µl. After a further incubation at ambient temperature of a least 30 min the signal was read on a Perkin Elmer Discovery fluorimeter.

The effect of IKK- $\beta$  inhibitors on primary synovial fibroblast mediator production was assessed as follows: primary cultures of human RSF were obtained by enzymatic digestion of synovium obtained from adult patients with rheumatoid 10 arthritis as previously described (Roshak *et al.*, 1996b). Cells were cultured in Earl's Minimal Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/mL streptomycin (GIBCO, Grand Island, NY), at 37°C and 5% CO<sub>2</sub>. Cultures were used at passages 4 through 9 in order to obtain a more uniform type B fibroblast population. For some studies, fibroblasts 15 were plated at 5 x 10<sup>4</sup> cells/mL in 16 mm (diameter) 24 well plates (Costar, Cambridge, MA). Cells (70-80% confluence) were exposed to IL-1 $\beta$  (1 ng/mL) (Genzyme, Cambridge, MA) for the designated time. Drugs in DMSO vehicle (1%) were added to the cell cultures 15 minutes prior to the addition of IL-1. Studies were conducted 3-4 times using synovial cells from different donors. RSF cellular 20 extracts were prepared from cells treated as described above. Briefly, human RSF were removed by trypsin/EDTA, washed, and harvested by centrifugation. Cellular extracts were prepared as previously described (Dignam *et al.*, 1983; Osborn, *et al.*, 1989). Briefly, at the end of the incubation period the cells (1x10<sup>7</sup>cells) were washed 2x in PBS without Ca<sup>2+</sup> and Mg<sup>2+</sup>. The resulting cell pellets were 25 resuspended in 20 uL of Buffer A (10 mM Hepes (pH 7.9), 10 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.5 mM.

Effect of IKK- $\beta$  inhibition on human monocyte stimulated eicosanoid and cytokine production was assessed as follows: monocytes were isolated from heparinized whole blood by double gradient centrifugation as previously described. 30 Isolated monocyte enriched PBMCs were then adhered to 24 well culture plates at 2 x 10<sup>6</sup> cells/mL in RPMI 1640 10% FBS (Hyclone, Logan, Utah) for 2 h. to further

enrich the monocyte population. The media was then removed, cells washed once with RPMI 1640, and 1 mL RPMI 1640 10% FBS was added to the wells. Test compounds are added to the wells with a final vehicle concentration of 0.05% DMSO. Monocytes were activated by the addition of 200 ng/mL endotoxin (LPS; *E. coli* serotype 026:B6)(Sigma, St. Louis, MO.) and incubated for 24 hrs. Cell-free supernates were analyzed by ELISA for TNF- $\alpha$  (EIA developed at SB), PGE<sub>2</sub> (Cayman Chemical, Ann Arbor, MI), and IL-8 and IL-6 Biosource International, Camarillo, CA). Viability of the cells was determined by trypan blue exclusion.

Effect of IKK- $\beta$  inhibitors on phorbol ester-induced inflammation was assessed as follows: the inflammatory response induced by the cutaneous application of phorbol ester (PMA) to the external pinnae of Balb/c mice has proven to be a useful model to examine multifactorial inflammatory cell infiltration and inflammatory alteration of epidermis. The intense inflammatory lesion is dominated by neutrophil infiltration, which can be easily quantified by measurement tissue concentration myeloperoxidase, an azurophilic granular enzyme present in neutrophils. In addition, the overall intensity of the inflammatory response can be measured by determination of ear thickness. Balb/c mice (n = 6/group) were administered drug treatment or vehicle followed by PMA (4 ug/ear). The mice were sacrificed 4 h. later, the ear thickness determined and NF- $\kappa$ B activation was monitored by I $\kappa$ B $\alpha$  western or EMSA analysis.

Effect of IKK- $\beta$  inhibitors on rat carrageenan-induced paw edema was assessed as follows: male Lewis rats (Charles River- Raleigh, NC) were housed and allowed free access to food and water, and weighed between 200-275g for each experiment. Compound or vehicle (0.5% Tragacanth (p.o.) or 10%DMSO, 25%DMA, 30% Cremophor(i.p.)) was administered 30 minutes to 1 hour prior to the carrageenan injection. Edema was induced by injection of 1% carrageenan in sterile dH<sub>2</sub>O (0.05ml/paw) into the plantar surface of the right hindpaw. Paw thickness was measured prior to administration of compound or vehicle, and again at 3 hours, to determine change in paw volume. Rats were euthanized by CO<sub>2</sub> inhalation and the right hindfoot was removed, immediately frozen in liquid nitrogen and stored at -80C for analysis.

To determine the effects of an IKK-2 inhibitor in the mouse collagen-induced arthritis (CIA) model, 12 male DBA/1 mice (20-22 grams) per treatment group were immunized on day 0 with a total of 100 uL of complete Freund's adjuvant (CFA) containing 200 ug of bovine type II collagen. On day 21 mice were boosted with

5 100 uL of phosphate buffered saline (PBS) containing 200 ug of bovine type II collagen (the 100 uL of collagen/CFA or collagen/PBS was injected subcutaneously into the tail). The IKK-2 inhibitor in vehicle, or vehicle alone, was administered intraperitoneally, twice daily, from days 1 through 40 (disease symptoms are evident beginning on days 25-28). Two additional treatment groups included the positive

10 control etanercept (Enbrel) (4 mg/kg, intraperitoneally, every other day), and the etanercept vehicle (PBS). Mice were scored daily, through day 50, for clinical symptoms (see below), and paw thicknesses were measured. In addition to the 12 mice per treatment group that were scored throughout the experiment, at several time points during the course of disease satellite mice (3-5 per treatment group) treated as

15 described above were utilized to measure cytokine/chemokine levels and p65 levels in the paw, the *ex vivo* antigen recall response by draining lymph node antigen recall response by draining lymph node

**Induction of arthritis:** AIA is induced by a single injection of 0.75 mg of

20 *Mycobacterium butyricum* (Difco, Detroit, MI) suspended in paraffin oil into the base of the tail of male Lewis rats aged 6-8 weeks (160-180 g). Hindpaw volumes are measured by a water displacement method on day 16 and/or day 20. Test compounds were homogenized in a suitable vehicle and administered by a suitable route. Control animals are administered vehicles alone. Two dosing protocols are

25 generally used: prophylactic dosing, which is initiated on the day of adjuvant injection and therapeutic administration, initiated on day 10 once inflammation has been established.

**Clinical scoring**

Each paw was assigned a score ranging from 0-4, based on the following criteria:

30 0 = no inflammation

1 = single swollen digit

2 = several swollen digits, mild paw swelling

3 = several swollen digits, moderate paw swelling

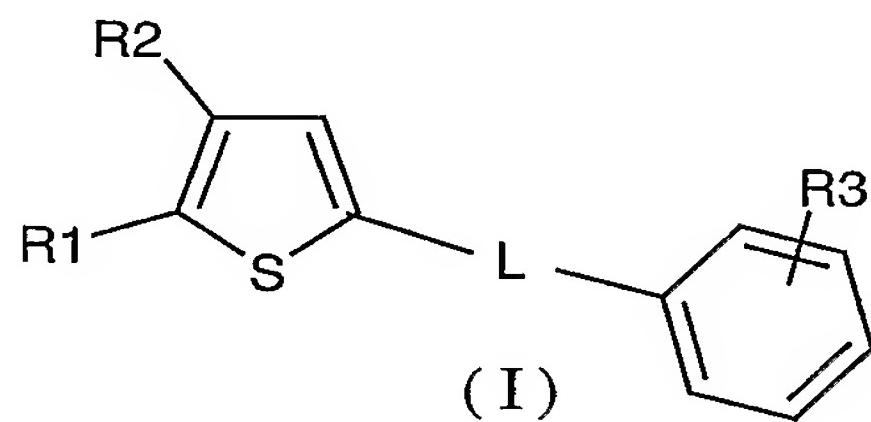
4 = all digits swollen, severe paw swelling

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the area can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A compound according to formula (I) hereinbelow:



5 wherein:

R<sub>1</sub> represents NR<sub>4</sub>R<sub>5</sub>;

R<sub>2</sub> represents CONH<sub>2</sub> or SO<sub>2</sub>NH<sub>2</sub>;

R<sub>3</sub> represents up to three substituents selected from the group consisting of halogen, C<sub>1-4</sub>alkyl, NH<sub>2</sub>, CF<sub>3</sub>, OCF<sub>3</sub>, O-alkyl, S-alkyl, CN, CHO, SO<sub>2</sub>-alkyl, and NO<sub>2</sub>;

10 R<sub>4</sub> represents H, C<sub>1-2</sub> alkyl;

R<sub>5</sub> represents C(=A)NHR<sub>6</sub>, COR<sub>7</sub>, or R<sub>6</sub>;

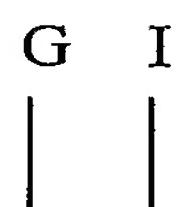
A represents O, S, or N;

R<sub>6</sub> represents H, or C<sub>1-2</sub> alkyl;

15 R<sub>7</sub> represents C<sub>1-2</sub> alkyl;

L represents a linker D-E-D such that

D represents a bond or C<sub>1-4</sub> alkyl;



20 E represents C = C, CONH, NHCO, COO, N, O, S, or  $\equiv$ ; and

G and I independently represent H, C<sub>1-2</sub> alkyl; or a pharmaceutically acceptable salt thereof, provided that the compound of formula (I) is not 2-[(aminocarbonyl)amino]-5-{[(4-chlorophenyl)methyl]oxyl}-3-thiophenecarboxamide.

25 2. A compound according to claim 1 wherein R<sub>2</sub> is CONH<sub>2</sub>.

3. A compound according to claim 1 wherein R<sub>5</sub> is C(=A)NHR<sub>6</sub>.

4. A compound according to claim 1 wherein A is O.

5. A compound according to claim 1 wherein E is



6. A compound according to claim 1 wherein the compound is selected from the group consisting of:

- 10 5-[(E)-phenyl]-ethenyl]-2-ureido-thiophene-3-carboxylic acid amide;  
5-[(E)-2-(4-Fluoro-phenyl)- ethenyl]-2-ureido-thiophene-3-carboxylic acidamide;  
5-[(E)-2-(4-Chloro-phenyl)- ethenyl]-2-ureido-thiophene-3-carboxylic acid amide;  
5-Phenethyl-2-ureido-thiophene-3-carboxylic acid amide;  
5-Benzyl-2-ureido-thiophene-3-carboxylic acid amide;
- 15 5-(1-Phenyl-ethyl)-2-ureido-thiophene-3-carboxylic acid amide;  
5-Phenylethynyl-2-ureido-thiophene-3-carboxylic acid amide;  
5-(4-Fluorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
5-(4-Ethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
5-(4-Methoxyphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;
- 20 5-(4-Chlorophenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide ;  
5-(4-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
5-(3-Trifluoromethylphenylethynyl)-2-ureido-thiophene-3-carboxylic acid amide;  
and
- 25 5-Acetylamino-thiophene-2,4-dicarboxylic acid 4-amide 2-[(3-chloro-phenyl)-  
amide]; or a pharmaceutically acceptable salt thereof.

7. A method of treating a disease characterized by pathological NF- $\kappa$ B activation comprising inhibiting the pathological activation by administering to a patient in need thereof an effective amount of a compound according to claim 1.

8. A method according to claim 7 wherein the disease is an inflammatory or tissue repair disorder.
9. A method according to Claim 8 wherein the disease is selected from the group consisting of inflammatory and tissue repair disorders, particularly rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease) osteoarthritis, osteoporosis and fibrotic diseases, dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage, autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkin's disease, cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome, and Ataxia Telangiectasia.  
15
10. A method according to claim 7 wherein said disease is COPD.
11. A method according to claim 7 wherein said disease is asthma.
- 20 12. A method according to claim 7 wherein said disease is rheumatoid arthritis.
13. A method according to claim 7 wherein said disease is dermatosis.
- 25 14. A method according to claim 7 wherein the disease is selected from the group consisting of: psoriasis, atopic dermatitis, and UV-induced skin damage.
15. A method according to claim 7 wherein the disease is selected from the group consisting of autoimmune diseases; tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, osteoarthritis, 30 osteoporosis, and Ataxia Telangiectasia.

16. A method according to claim 7 wherein said disease is an autoimmune disease.
  17. A method according to claim 9 wherein the autoimmune disease is systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, or ankylosing spondylitis, diabetes
  18. A method according to claim 7 wherein the disease is cancer and/or cachexia.
  19. A method according to claim 7 wherein the cancer is Hodgkin's disease.
- 10
20. A method according to claim 7 wherein the disease is inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS).
  - 15 21. A method according to claim 7 wherein the disease is AIDS.
  22. A method according to claim 7 wherein the disease is adult respiratory distress syndrome.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/17385

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 333/36; A61K 31/38  
 US CL : 549/68, 69; 514/447

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 549/68, 69; 514/447

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 CAS ONLINE-structure search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	Database HCAPLUS on STN, AN 2003:97415, GRIFFITHS, D. et al. 'Preparation of ureido-carboxamido thiophenes as inhibitors of IKK2 kinase'. CA 138:153430, WO 2003010163, 06 February 2003, Abstract.	1-22

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T".	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

23 October 2003 (23.10.2003)

Date of mailing of the international search report

29 OCT 2003

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